REMARKS

Claims 1-13 and 16-40 were examined and reported in the Office Action. Claims 1-13 and 16-40 are rejected. Claims 1-7, 9-10, 12-13, 16, 19, 22-23, 27, 29, 31-32, 34-37 and 40 are amended. Claims 1-13 and 16-40 remain.

Applicant requests reconsideration of the application in view of the following remarks.

I. In the Drawings

Applicant has added new Figures 19-21. Figure 19 illustrates a prism dispersion means. Figure 20 illustrates diffraction grating dispersion means. Applicant's original specification discloses dispersion means on page 5, lines 5-6. Applicant lists examples of the dispersion means on page 16, lines 2-8. In particular, the fluorescence light beams exiting the capillaries C, which are collimated, may be directly transmitted to one or more intermediate prisms or else to a diffraction grating in order to spatially separate the various wavelengths emitted and to separate them on an array of photodetectors. Applicant notes that the original specification disclosed the features illustrated in new figures 19 and 20. Applicant also notes that the features added to figures 19 and 20 are limitations in the claims not previously illustrated. Applicant notes that the amendments to the drawings only helps in understanding the claimed subject matter.

Figure 21 illustrates means for applying pressure in the form of a pump. Applicant's original specification discloses the means for applying pressure on page 5, lines 2-4. In addition, Applicant's original specification discloses an example of such means on page 11, lines 31-34. In particular, Applicant's original specification discloses "the separating matrix (a gel or other material) is injected into the capillaries by means of a pump which allows pressure to be applied in the detection cuvette." Applicant notes that the location of the supply of the separating matrix in the cuvette is of no importance. The separating matrix can be filled anywhere in the cuvette. Additionally, it is well known in the art of electrophoresis to connect the detection cuvette with means for applying pressure. Applicant also notes that the feature added to figure 21 is a limitation in the claims not previously illustrated. Applicant notes that the amendment to the drawings only helps in understanding the claimed subject matter.

Applicant encloses six documents which illustrate means for applying pressure, such as pumps that are used to apply pressure in the separating medium asknown in the art.

Therefore, no new matter is added. Approval is respectfully requested.

II. 35 U.S.C. §112, First Paragraph

It is asserted in the Office Action that claims 9, 10, 31 and 32 are rejected under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. Applicant has added new figures 19-21 to assist in understanding the claimed subject matter and asserts above that the claimed limitations are originally disclosed in the specification well enough for one of ordinary skill in the art to be able to make and or use the claimed invention. Regarding the new figures, the figures illustrate the location of the dispersion means and also the means for applying pressure (i.e., pump P). These features are disclosed and are added to original figure 2 to assist in understanding the claimed invention.

Accordingly, withdrawal of the 35 U.S.C. §112, first paragraph rejections for claims 9, 10, 31 and 32 are respectfully requested.

III. 35 U.S.C. §112, Second Paragraph

It is asserted in the Office Action that claims 1-13 and 16-40 are rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention. Applicant has amended the claims to overcome the 35 U.S.C. §112, second paragraph rejections.

Accordingly, withdrawal of the 35 U.S.C. §112, second paragraph rejections for claims 1-13 and 16-40 are respectfully requested.

IV. Claims Not Rejected Over Prior Art

Applicant notes that claims 1-13 and 16-40 are amended to overcome the 35 U.S.C. § 112, first and second paragraph rejections and are not rejected over prior art. Applicant presumes the arguments and amendments presented in the response mailed July 24,2003 overcame the prior art rejections as the Office action made no specific mention.

Applicant respectfully asserts that claims 1-13 and 16-40, as it now stands, are allowable for the reasons given above.

APPENDIX

Document Number	*Issue Date*	*Patentee or Applicant*
5,164,055	11/17/1992	Dubrow
6,352,633	3/05/2002	Liu et al.
6,027,627	2/22/2000	Li et al.

Non-Patent Literature Documents
Goetzinger, W., et al. "High Molecular Mass Emulsion Polymerized Linear Polyacrylamide", Electrophoresis 19 (1998) 242-243.
Scherer, J. R., et al., "Rotary Capillary Array Electrophoresis Scanner", Electrophoresis 20 (1999) 1508-1517.
Behr, Sven, et al., "A fully Automated Multicapillary Electrophoresis Device for DNA Analysis", Electrophoresis 20 (1999) 1492.

greatly simplified if LPA were available in powdered form. Such a powder could be easily handled, with a practically unlimited shelf life.

Commercially available powdered LPA was initially examined but found to be unsatisfactory as a solution for DNA sequencing [13]. The polymer was typically contaminated with impurities from the production process that reduced the electrophoretic separation or diminished the fluorescence detection of the sequencing reaction products. These effects were found to occur with polymer molecular mass standards as well, which, in addition, are very expensive. Moreover, at times, a significantly reduced lifetime for the capillary coating was observed. Indeed, most researchers, using LPA, freshly prepare their own matrix by solution polymerization. However, problems with reproducibility of the polymerization have been reported, and the resulting polymers have not been accurately characterized.

In this paper, we introduce an alternative procedure for preparing high molecular mass LPA in powdered form, namely inverse emulsion polymerization [14]. In this method, a highly concentrated aqueous solution of acrylamide is dispersed in an oil phase [15, 16]. The polymerization occurs in small water/monomer droplets, which act as micro-batch reactors. In contrast to polymerization in aqueous solution, the water/oil emulsion has low viscosity and good heat transfer, which allows the use of a highly concentrated monomer solution for the polymerization. The high concentration of monomer in the water droplets leads to a high molecular mass polymer. The procedure allows the preparation of large amounts of LPA as a white powder with the desired properties for separation of DNA sequencing reaction products. In this work, the polymers are characterized by size exclusion chromatography (SEC) and viscometry. In addition, procedures to prepare and handle solutions made from high molecular mass LPA are described. The successful application of these solutions to high performance DNA sequencing by CE is demonstrated.

2 Materials and methods

2.1 Chemicals and reagents

Acrylamide, urea, ammonium peroxodisulfate (APDS) and TEMED were ultrapure grade from ICN Biomedicals (Costa Mesa, CA). The Span 80 emulsifier and petroleum special with a boiling range from 180–220°C were purchased from Fluka Chemicals (Buchs, Switzerland). Polyacrylamide molecular mass standards were obtained from American Polymer Standards (Mentor, OH). Dye Primer Cycle Sequencing Ready Reaction Kit with (-21) universal dye primer set (Cat. No. 402135) was from Perkin-Elmer/Applied Biosystems Division (Foster City, CA), and M13mp18 ssDNA from New England Biolabs (Beverly, MA).

2.2 Polymerization of acrylamide and preparation of the LPA matrix solution

The polymerization was performed according to a protocol [15] with slight variations. In a Pyrex reaction flask, a 40% w/w solution of acrylamide was dispersed in a solution of 2.4% SPAN 80 in petroleum special to a volume ratio of 1:1. Dispersion was performed by an overhead stirrer with a propeller blade at 500 rpm. APDS and TEMED were used to catalyze the reaction, both at a final concentration of 0.005% w/v. To remove oxygen, the entire dispersion was purged continually with nitrogen, and then the polymerization reaction was performed at 35°C for 16 h. Polyacrylamide was obtained by precipitating the emulsion with acetone under vigorous stirring. The precipitate was washed several times with acetone on a Buechner funnel, and residual solvent was removed under oil-pump vacuum on a rotary evaporator. To prepare LPA matrix solutions for DNA sequencing, the dry polymer, denaturant (usually urea), buffer concentrate, and water were added to the desired concentrations in a glass jar and then slowly stirred with a magnetic bar. The solutions were usually completely homogenized overnight and ready for use.

2.3 Characterization of LPA

SEC was performed with an HPLC pump (Bischoff, Leonberg, Germany) equipped with an analytical pump head. Samples were injected with a Rheodyne valve (Alltech, Deerfield, IL). The polymer powder was dissolved at 0.1% w/w concentration in 0.1 M sodium sulfate. After dilution to 0.025% w/w in elution buffer, the samples were passed through a 5 µm filter prior to injection. A Shodex KB 806 (300 × 7.8 mm) column with an exclusion limit of 20 MDa was used for characterization of the high molecular mass LPA samples. Similar results were obtained with an Ultrahydrogel 2000 column (Waters, Milford, MA); results not shown). The eluent, 0.1 M sodium sulfate, was maintained at a flow rate of 0.5 mL/min. The injection volume was 25 µL and detection was performed at 214 nm with a Waters UV detector. The viscosity of solutions containing 2% w/w LPA made by emulsion polymerization were determined in a Brookfield Viscometer model "LVDV I+" (Stoughton, MA). For polymer characterization, the viscometer was equipped with a small sample adapter, and a spindel type 25/13R was used for the measurement at a rate of 6 rpm, resulting in a shear rate of 1.32/s. For the studies on the influence of shear forces on polymer molecular mass distribution, variable shear rates up to 40/s were applied. The viscosities were measured at 25°C. The time required for the replacement of LPA matrix solution from a capillary at a given pressure was determined using an in-house design. A 45 cm long, 100 µm ID capillary filled with 2% w/w LPA solution, containing TTE buffer (50 mM Tris, 50 mM TAPS, and 2 mM EDTA) and 7 M urea, was connected to one meter, 1/16" OD PEEK tubing filled with the same LPA solution. The tubing served as a reservoir with a fresh polymer solution. To make the replacement visible, the LPA solution in the capillary was stained with Coomassie Blue. A nitrogen gas tank equipped with a regular manifold was connected to the other tip of the PEEK tubing, and various pressures were applied to the LPA solution at room temperature. The replacement process was monitored using a Spectra 100 variable wavelength detector (ThermoQuest, Mountain View, CA).

document 1 : W. Goetzinger et al., Electrophoresis 19 (1998) 242

eased data rate is only 16 384 data points per second (1022 data positions × 4 colors × 4.008337 Hz). A rotation speed of 4 rev/s was chosen to ensure that all the DNA fragments passing through the 20 μm long detection zone of the capillary are interrogated.

2.1.5 Signal to noise optimization

Rotary scanning enables rapid and smooth beam motion that is essential for high-speed interrogation of a large number of capillaries. The consequence of these conditions for the limiting signal-to-noise ratio can be considered with reference to theories of fluorescence detection optimization [27]. In our application, the detection zone of the 340 mW (8.5×10^{17} photons per second) laser beam is approximately a 20 × 20 μm area and the transit time of the 20 μm beam past a pixel is ~16 μs at a scan rate of 4 Hz. For fluorescein this gives a dimensionless excitation intensity (k) of ~0.3 and a dimensionless transit time (τ) of ~0.1 based on a photodestruction quantum yield of 2.7×10^{-5} [27]. These parameters are better than those used for our linear scanners ($k = -0.008$, $\tau = -13$) and are close to the optimum for laser-excited fluorescence detection [27]. This results in near-optimal interrogation of all of the analytes illuminated by the beam. Furthermore, the velocity of an ~300 base sequencing fragment is ~100 μm/s; the 4 Hz data acquisition rate therefore interrogates ~80% of the fragments in a typical electrophoretic band. The width at half-height for a 300 base band is ~4 s, which leads to 16 data points over the half-width. Fragments of smaller size will be somewhat under-sampled while larger fragments will be oversampled. Nevertheless, the effective duty cycle of this high-speed scanning system is within a factor of two of that available in continuous illumination and CCD imaging detection [24–26] and the photophysical properties are near optimal. The limits of detection of this system were determined by flowing fluorescein at varying concentrations through the capillaries. Scanning at 2 Hz with 600 mW of laser power and a flowing fluorescein solution, we find that the difference signal (510–530 nm) between 10 pM fluorescein and water can be observed with a signal-to-noise ratio of 2. We determined the optimum laser power by observing fluorescence from a capillary filled with 1 nM fluorescein solution in 1 × TBE and increasing the laser power until the signal reaches a plateau. At these power levels (typically ~300 mW) there is no degradation of signal in a stationary fluorescein solution when scanned at 2–4 Hz. This indicates that at these rotation velocities, the illumination time is below the photodestruction time and that the signal is limited by ground state depletion and the number of fluorescent molecules present. Diffusion between scans replaces molecules as fast as they are destroyed.

2.2 Capillaries, sieving matrix and samples

The fused-silica capillaries (50 cm long, 75 μm ID, 180 μm OD) were obtained precoated with acrylamide from Polymicro Technologies (Phoenix, AZ) courtesy of Molecular Dynamics. These capillaries had been surface-treated using a Grignard reaction and coated with 3% acrylamide according to the procedure described by Dolnik *et al.* [28]. The capillaries were cut and bundled into groups of 32 as described above. The windows in the capillaries were burned 8.1 cm from the anodic end using hot concentrated sulfuric acid. We used a replaceable linear polyacrylamide (LPA) sieving matrix prepared according to the procedure of Carrilho *et al.* [13] with slight modifications. A 6% solution of acrylamide in 10 mL of water was degassed under helium for half an hour followed by addition of 5 μL of 10% TEMED (Bio-Rad, Hercules, CA) and 10 μL of 10% APS (ammonium persulfate; Bio-Rad) at 0°C. The polymerization was allowed to proceed for 24 h at 0°C. The polymerized gel was diluted to a final concentration of 3% in 7 M urea and 50 mM Tris, 50 mM *N*-tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid (TAPS) and 2 mM EDTA buffer. DNA sequencing samples were synthesized using M13mp18 single-stranded templates, cyanine donor energy transfer (ET) primers [29] and Thermo Sequenase (Amersham Life Science, Cleveland, OH) following the Sanger dideoxy method [30]. Four DNA sequencing reactions were set up, one for each dye/ddNTP pair. C10R110, C10G, C10T and C10R primers were used to label reactions terminated with ddCTP, ddTTP, ddGTP and ddATP, respectively. For each reaction, 1 μL of the pre-mix from the Thermo Sequenase kit was mixed with 0.4 pmol of the primer and 0.2 μg of the template DNA. Twenty cycles (95°C for 45 s, 45°C for 30 s and 70°C for 45 s) were performed, followed by ethanol precipitation. The precipitated DNA was dissolved in 4 μL of 70% deionized formamide for loading.

2.3 Sieving matrix loading

The capillaries are filled with replaceable LPA by loading the matrix into the well (Fig. 2 K) and forcing it into the capillaries with He gas under pressure. The well is filled with replaceable sieving matrix using a syringe. The upper and lower lucite plates are bolted together and the trough is pressurized to fill the capillaries. The unit has been pressure-tested to withstand 1000 psi. In the experiments performed here, the capillaries were filled with 3% LPA at ~400 psi in 10 min. After filling the capillaries with matrix, the lower pressure chamber is unbolted and lowered and two half-wells containing running buffer and electrodes are bolted to the upper adaptor plate (Fig. 2H). The polymer-filled capillaries are preelectrophoresed for 10 min at 100 V/cm. Our electrophoretic separa-

document 2: J.R. Scherer et al., Electrophoresis 20(1999) 1508

laries to provide rapid and efficient excitation and detection of the fluorescently labeled fragments within the cylindrical capillary array.

2 Materials and methods

2.1 Instrumentation

2.1.1 Rotary capillary array scanner

Figure 1 presents an overview of the four-color rotary CAE scanner. The four-color confocal detection system in the upper part of this figure is the same as that used in our planar CAE system [20]. The 488 nm argon ion laser excitation beam is deflected downward into the scanner head with a dichroic beam splitter. A 45° diagonal mirror located in the central rotor of the scanner head directs the beam horizontally to an objective that focuses the beam into the capillaries. Over one thousand capillaries are held in grooves on the outside of two ~4" diameter cylinders. For ease of mounting, the capillaries are grouped into bundles, each containing 32 capillaries. Fluorescence from the capillaries is collected by the objective and passed to the four-color confocal detection system. This layout permits facile detection of fluorescence from 1000 capillaries with a single compact scanner.

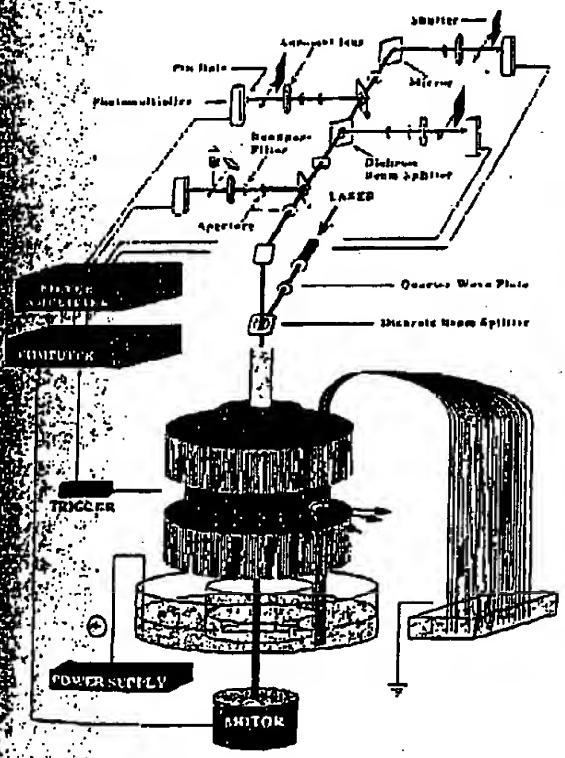

Figure 1. Schematic representation of the 1000 capillary rotary scanner and the four-color confocal fluorescence detection system.

Figure 2 presents a photograph of one quadrant of the scanner head with capillary bundles in the first and fourth positions (A). A microscope objective (E) (fl 9 mm, NA

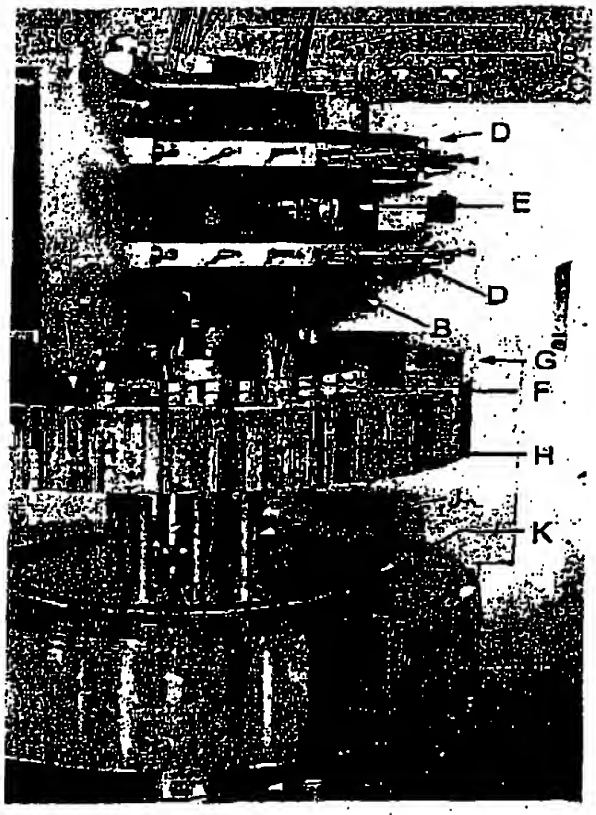

Figure 2. Photograph of one quadrant of the rotary scanner with the high-pressure reservoir detached. (A) Capillary bundles in the first and fourth group positions of the quadrant; (B) upper and lower grooved cylinders; (C) supports that connect the two cylinders; (D) U-shaped delrin blocks that retain the capillaries; (E) objective attached to the central rotor; (F) PEEK fitting screwed into the high-pressure adaptor plate (H); (G) bolting plates for the 2nd and 4th quadrants; (I) central shaft attaching the motor (M) to the central rotor; (J) bolts that secure the inner part of the high pressure reservoir to the adaptor plate (H); (K) the inner well for loading the matrix and the O-rings that seal the adaptor plate; (L) high pressure Lucite block.

document 2

mass:charge ratio which normally hinders nucleic acids to be separated in free solution. However, more development is necessary to achieve the high resolution necessary for DNA sequencing.

3.2 System overview and description

Based on the above-mentioned considerations, we have assembled a fully automated, stand-alone multicapillary electrophoresis prototype that is capable of automatically processing up to 40 microtiter plates without human intervention. The self-contained, fully enclosed system with interlocks for the laser and the high voltage power supply ensure a maximum of safety. The device is specialized for high throughput DNA analysis in a robust and routine way, but can be adapted for other applications as well. Here, we give a detailed description of the main parts of the system.

3.2.1 Detection area

A sketch of the system, discussed in detail in the following sections, is shown in Fig. 3. The capillary array consists of 96 capillaries of 58 cm length, with the detection windows at a distance of 38 cm from the injection end. In the window area, the capillaries are fixed in individual U-shape grooves (0.4 mm wide, 0.5 mm deep, separated by 0.1 mm thick walls) over a length of 2 cm. The grooves are engraved in aluminum blocks ("capillary holder"), each block with 16 grooves. The six blocks are aligned on a base plate and covered by a thin metal frame. The central slit in the frame gives access to the array of windows. Thus, the whole array of capillaries is subdivided into six arrays of 16, which can be replaced individually. This seems to be a good compromise, as it will hardly be necessary to change all capillaries at the same time. On the other hand, changing individual capillaries might be difficult.

3.2.2 Injection area and autosampler

From the detection window towards the injection end, the capillaries spread out into the 12 × 8 two-dimensional array of a standard microtiter plate. They are contained in an isolated compartment, which can be thermostated by air circulation up to 50°C. Through the last few centimeters, up to one cm from the end, the capillaries are guided by peek tubing and isolated from the platinum electrodes which are fixed about 1 mm apart and parallel to the capillary. All inlet electrodes are connected to common ground. Both capillaries and peek tubing, together with the electrodes, are held by a base plate, which itself is

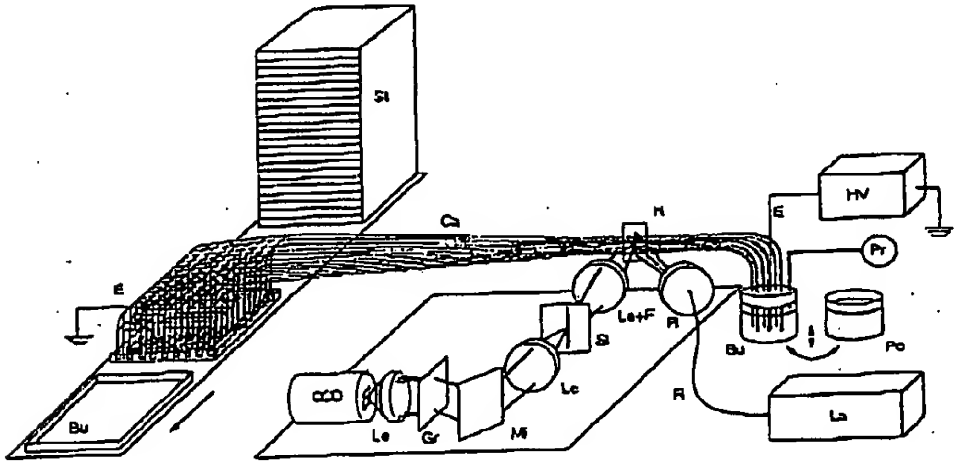

Figure 3. Scheme of the automated multicapillary electrophoresis device. The capillary (Ca) array consists of 96 capillaries, subdivided into six arrays of 16. In the window area, they are fixed in the capillary holder (H) in individual U-shaped grooves. The light from an argon-ion laser (La) is guided through an optical fiber (Fi) to the line generator (Pl), which spreads out the collimated light in one dimension and shines a thin line with a uniform intensity profile across the whole array of capillary windows in an angle of about 30°. The emitted light from the capillaries is collected through a filter with a 50 mm $f = 1/1.2$ lens (Le+F) and focused onto the slit (Si). A second lens (Le) collects and collimates the emitted fluorescence. The light is reflected by a mirror (Mi) and passed onto the holographic transmission grating (Gr). A third lens collects this light and produces the final image on the CCD camera (CCD). The microtiter plates containing the samples are stored in a stacker (St) and brought to the injection area by a transport unit. After injection, the sample plate is replaced by a second plate filled with electrophoresis buffer (Bu) and the high voltage (HV) is applied to the electrodes (E). After the run, the outlet vessel with buffer is replaced by the polymer reservoir (Po), and the chamber is pressurized with air (Pr: pressurized air supply).

document 3 : Sven Behr, Martin Mätzig, Electrophoresis 20 (1999) 1492